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Male breast cancer (MBC) is rare, with an incidence rate of 0.5-1/100,000 per year. It has a significant familial component and is observed in combination with female breast cancer in BRCA2 kindreds, while it is rarely observed in BRCA1 kindreds. The objective of this grant is to study a series of unselected population-based MBC cases and their relatives to characterize the role of BRCA2 in MBC and to estimate the attributable risk of MBC due to BRCA2 mutations. At the end of one year, we have collected family history data and DNA samples on 78 MBC cases and paraffin-embedded tissue on 16 of those. Of the 78 MBC cases, 41 (53%) have a family history of breast cancer in a first or second degree relative. Sixty six MBC cases have been screened for mutations in 38 of the 79 BRCA2 amplicons. Three predisposing, truncating mutations, one missense mutation of unknown significance, and one polymorphism have been identified. The missense mutation had been identified previously in an early-onset breast cancer family which we have studied. The polymorphism occurs with a frequency of 24% and will be useful in constructing the haplotypes of these MBC cases.

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FOREWORD

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Susan Newhauser 7-11-97

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Annual Progress Report
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Annual Progress Report
Grant DAMD17-96-1-6266
Period: June 15, 1996-June 15, 1997

Introduction:

Breast cancer in men was first described in the 14th century and the first familial case was reported in 1889 (Kozak et al., 1986). Breast cancer in men is rare, accounting for only about 1% of all breast cancers and 1% of all cancers in men (Boring et al., 1994). It has an incidence rate of 0.5-1/100,000 men per year, which is 100-fold less than in women. However, male and female breast cancers are indistinguishable histologically (Thomas et al., 1992), and clinically, where there are the same patterns of invasiveness, estrogen and progesterone receptor expression, and response to treatments (Jaiyesimi et al., 1992).

Many risk factors for male breast cancer (MBC) have been postulated and include a family history of both male and female breast cancer. Little is known about the genes responsible for genetic predisposition to MBC, with mutations in the androgen receptor (AR) gene and BRCA1 unlikely to account for a significant amount of predisposition to MBC. In contrast, BRCA2, (Stratton et al., 1994; Wooster et al., 1994; 1995) appears to be responsible for a greater proportion of MBC. Easton et al. (1997) calculated the lifetime risk of breast cancer in male BRCA2 carriers to be 6% based on analysis of two large families which had known ascertainment. Assuming a gene frequency for BRCA2 similar to that for BRCA1, a significant fraction of all MBC (10-15%) could be attributed to germline mutations in BRCA2. More accurate estimates await examination of BRCA2 in an unselected series of MBC cases.

The objective of this proposal is to study a series of unselected MBC cases to characterize the role of BRCA2 in MBC and to estimate the attributable risk of MBC due to BRCA2 germline mutations. Secondly, identification of BRCA2 mutations in these MBC cases will allow us to ascertain a set of high-risk families which can be used to further examine the genetic epidemiology of BRCA2-related cancers, including identifying underlying risk factors related to incidence of breast cancer in mutation carriers and risks of other types of cancers resulting from these mutations.

Body:

Our goals for the first year included ascertainment of MBC cases and for cases for whom we had tissue blocks, characterization of loss of heterozygosity (LOH) surrounding BRCA2 and fine-structure haplotype construction. We did not plan to screen for mutations until year 3. However, with the isolation of BRCA2 at the end of 1995, we began to screen for mutations using single strand conformational analysis (SSCA) in year 1. We will begin the haplotyping and LOH analysis in Year 2.

Progress in ascertainment of male breast cancer cases: MBC cases are being ascertained in Utah through the Utah Cancer Registry (UCR). The UCR is the agency designated to record all cancer diagnosed in the state. Therefore, the samples are all population-based. MBC cases also are being ascertained from Memorial Sloan Kettering Cancer Center (MSKCC) and are therefore clinic based. From a previous study we did examining colorectal cancer cases, we found that there were no statistical differences between population-based and clinic-based samples for age-of-onset, ethnicity, histoprognostic indicators, etc. As we accumulate more data, we will compare these two groups to determine if there are any differences.

We currently have blood samples and family history information from 78 MBC cases. DNA has been extracted from all blood samples. Fifty cases were ascertained from the UCR and 28 cases from MSKCC. Of these 78 MBC cases, 41 (53%) have a family history of breast cancer in first or second degree relatives. Of those 41 MBC cases with a family history, 20 (49%) only have 1 relative with breast cancer. We currently have tissue blocks on 16 cases. DNA samples have been collected from at least one first degree relative for 28 of the cases.

The MBC cases have been more difficult to ascertain than we had anticipated, as approximately 50% of the cases in the UCR prior to December 1995 either had addresses which were unknown or were deceased. We currently have 11 letters pending at the UCR for permission to contact the MBC cases from diagnoses prior to December, 1995. We have amended our IRB protocol to include rapid-reporting of recently diagnosed cases in order to obtain a larger response rate. We just received permission to send out letters to MBC cases diagnosed in 1996. In addition, we are applying to the Idaho Cancer Registry to ascertain MBC cases diagnosed in Idaho from 1992 to the present (we estimate 15-25 cases).

Progress in screening for BRCA2 mutations. BRCA2 was isolated in December of 1995 (Wooster et al., 1995). Sequence information is available and we have designed 79 primer sets to amplify all coding regions and intron/exon boundaries of the BRCA2 gene. We are in the process of redesigning some primer sets which do not result in a strong signal following PCR amplification. We are screening for mutations using SSCA, followed by sequencing of variants to identify the exact mutation. We have completed screening 38 of the amplicons on 66 MBC cases. We have identified three frameshift mutations (4075delGT, 4360ins5, and 6174delT) resulting in truncation of the protein. The 6174delT mutation, previously identified as a recurrent mutation in individuals of Ashkenazi Jewish descent (Neuhausen et al., 1996), has been identified in three Jewish MBC cases from MSKCC. We just identified the 4306ins5 mutation and need to confirm the size and actual sequence of the insertion. The missense mutation (D1420Y), observed in one MBC case, had been seen previously in one of our early-onset female breast cancer families. Missense mutations are difficult to interpret as to whether they are predisposing to cancer. Various features may indicate a biologically deleterious effect such as 1) a low frequency of the variant in a control group; 2) cosegregation of the variant allele and disease; and 3) lack of similarity between the normal and the variant amino acid; and 4) location in putative functional domains. We will examine the frequency of this missense mutation in a set of 100 (200 chromosome) unrelated DNA samples and examine cosegregation with breast cancer in the family in which we have observed this mutation. The change in the amino acid is from a polar charged molecule to a neutral molecule. Lastly, a polymorphism (K1132K) which occurs with a frequency of 24% in this set of MBC cases was observed and may be useful for haplotype construction. Two other variants have been identified by SSCA, but sequencing has not yet been performed to identify the mutation. The number of individuals and amplicons examined is small, yet in this limited set, we have observed a predisposing mutation rate of 8% (5/66).

During the next year, our primary focus will continue to be to ascertain MBC cases and to screen for mutations using SSCA in additional amplicons and with newly ascertained MBC cases. In addition, we will begin to assess LOH for MBC cases for whom we have tissue blocks and to construct a fine-structure haplotype for those MBC cases who either exhibit LOH surrounding BRCA2 and/or for whom we have DNA samples of close relatives.

Conclusions:

These preliminary results are suggestive that a proportion of MBC, even in the absence of a significant family history, is due to BRCA2. There are not yet sufficient data to draw any conclusions regarding the mutation spectrum or prevalence of BRCA2 in MBC. Because the risk of MBC is small, most BRCA2 families will present without a male. Through ascertainment of MBC probands with a family history, we can identify additional women in BRCA2-linked families who have a high (>80%) lifetime risk of the disease, determine what the actual risk is to these women and men, and compare families with and without MBC. The cohorts identified in this study can be followed in future studies with respect to the interactions among genetic effects, known epidemiological risk factors, and potential modifier genes.

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